II.

Cytological aspects of the Virus diseases in Plants.

by

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A quarter of a century has elapsed since IWANOWSKI (19) opened a new era in the history of the virus diseases by his cytological expositions. But the represention of his findings in the diseased cells of tobacco moasic was so sceptic that it dit not awake the enthusiasm of scientific workers — the chief idea lurking behind their minds being that it was an enzymatic disease or a disease due to an ultra-microscopic, filterable organism. And even today there is quite a number of advocates who hold tenaciously to either of these opinions.

Much interest was awakened when LYON (32) observing a vacuolate organism (a body very similar to the one found by IWANOWSKI in mosaic diseased tobacco) in the Fiji disease of sugar cane put forward his view as to its causal nature. But KUNKEL (25, 26, 27), working on the Fiji disease of sugar cane, and Hippeastrum and corn mosaics and confirming the presence of such vacuolate bodies in them, has creditably tried to propagate this causal theory. A stimulus thus being obtained, fascinated workers were not lacking. Here is no space to discuss the causal, effective or neutral status of any such bodies. A general review of the cytological work done on the problem is to be taken here as whatever may the nature of the said agent be, „the question remains primarily a cytological one" (Cowdry, vide page 114, Filterable Viruses).

Vacuolate bodies. Among the intracellular inclusions found so far in the affected cells of such diseases these vacuolate bodies may be said to be of prime importance. Their presence is evidenced every now and then in different plants affected with similar diseases. They have been so far discovered in sugarcane (3, 4, 26, 32, 33, 40), Hippeastrum (3, 18, 27, 38), corn (3, 25), wheat (39), Eucharis (3), Sorghum (3), and Soudan grass (3), amongst the monocotyledons; in dicotyledons they have been known to occur in tobacco (6, 7, 14, 15, 17, 19, 30, 44, 50, 53), potato (17, 54), Dahlia (16), *Phytolacca decandra* (53), *Evonymus japonicus* (53), Chinese cabbage (27), strawberry (46), sugar beet (49), grape vine (47), sandal and red periwinkle (41). Some attempts were made to distinguish them according to the cotyledonous

nature of the host (4). They are so strinkingly alike in all these plants that such distinction is considered quite unnecessary.

Such contradictory evidences are put forward as to the position of these vacuolate bodies in different tissues of plants that one would feel rather at a risk to define an exact place for them. But the *ensemble* of all workers gives the idea about their presence in all the infected tissues of such plants. So far only one body has been found to be karyooikonally present in dahlia by GOLDSTEIN (16). Their number in cells is very variable. The cellular plasma-like substance of these bodies approaches more that of mitochondria (30, 47).

The form of these vacuolate bodies is round, oval, pearshaped, irregular or amoeboid. Sometimes a veillike structure is attached to them (25). No nuclear structure has been observed though chromidial granules may be said to be invariably present in them. Fat globules have been discovered in Hippeastrum bodies (3, 18). But the main structure of them is their extremely *vacuolate* nature. The number of these vacuoles may vary from one to many according to the size and age of these bodies. Pseudopodia have been observed (14, 15, 25, 26, 27, 30). Occasionally a whole body can be seen to prolong itself into a long pseudopodiumlike protuberance (30, 39). Division, encystment, sporulation and conjugation do exist (15, 25, 26, 30, 40). Germination from cysts is reported (40).

The above characteristics of these vacuolate bodies are so convincing of their animal, and parasitic and consequently causal nature that several times attempts have been made to designate a name for them (33, 40). Recently a general review of the nomenclature was made and the term **Vacuolarium** has been proposed for the genus (30). Some granular bodies found especially in the epidermal tissue have been grouped under chlamydozoa and proposed as the causal organisms (34, 35, 44, 46, 51). A nomenclature was also created (*Strongyloplasma Iwanowski*) by PALM (44). Recently these bodies have been explained as a phase in the life history of the vacuolate bodies (30, 55) and the artificial nomenclature is consequently suppressed.

A very interesting organism has been described (13) in mosaic-affected plants of *Hippeastrum Johnsonii*, pepper, tomato, squash, strawflower and dahlia. This organism was found in other parts of the infected plant (veins and adjacent mesophyll cells) twenty-four hours after inoculation. Spore formation was also seen. GOLDSTEIN (16) has taken these organisms as crushed vacuolate bodies. These organisms are distinctly flagellate, have a nuclear structure, form peculiarly

typical spores and may be said to approach more definitely the swarmspores of a myxomycete. Plasmodium and swarmspores are not uncommon in mosaic infected and healthy plants (20) and very often mistakes are committed by taking them as causal organisms. A clear evidence as to the nonpathogenicity of these organisms has been lately proved (31).

The extra bodies. Some spindle shaped, striped bodies were seen in connection with the other bodies in the stripe disease of *Hippeastrum equestre* (3). More regular and spherical bodies with a definite membrane have been mentioned as associated with these x-bodies (a term created by GOLDSTEIN) in the root tips of sugar beet with curly top (49) and strawberry with xanthosis (46). Nothing can be explained for the present about the nature of these extra-bodies.

It will be noted that a complete lack of such bodies has been announced in the calico of tobacco, and streak and leafroll of potato (17).

Crystalloid material. Certain crystals are a constant association of tobacco mosaic number 1. (6, 7, 14, 15, 17, 19, 22, 30, 50, 53). Albuminoid in nature (22), they are represented in polyhedral, striated and crescent forms according to their plane of vision and age of the cell. Owing to their constancy in this particular mosaic they are included in the index of tobacco mosaic number 1 along with the specific vacuolate body called *Vacuolarium Iwanowski* (30). Crystals of the same nature but of spindle form have been proposed as distinguishing marks of the mosaic and intercostal mosaic of potato (22). Interesting will it be to know that vacuolate bodies were already observed in several potato virus diseases. These crystal formations are the albuminoid secretions given out by these bodies in certain plants (30).

Crystalloid material has also been observed in nuclei of certain mosaic diseased plants (17). The nature of these intranuclear crystals is very likely to be the same as the other crystalloid material. A confirmative work on the point is yet wanted.

Flagellate bodies. At the end of 1922 NELSON (42) published his observations on some biflagellate structures in the phloem cells of mosaicinfected bean and clover, and trypanosome-like bodies in the mosaic of tomato and leafroll of potato. A theory has long been prevaling about the location of the ultra microscopic causal organisms in

those parts of plants affected with such maladies. Failing to find such bodies in healthy plants and bearing in mind the analogy of like organisms in Euphorbaceous plants he exposed his theory as to the causal nature of these organisms of the virus diseases. PETRI (45) discovering a spiral body in the leafcurl disease of vine, declared it as the probable cause of the malady.

It turned out that such biflagellate forms had already been figured by STRASBURGER (56) and others in Leguminous plants and a complete lack of any such structures was pointed out in the cucurbitous plants. A close search to verify the results of NELSON was, therefore, made by using healthy material. Certain authors have in their investigations evidently mistaken the nuclei or degenerated nuclei for the findings of NELSON and cannot here be dealt with (21, 29). These bodies are found in cells in which the nuclei are conspicuous. They may also be found surrounding the nuclei (42).

However, later workers have left no doubt as to the non-organismic nature of these bodies (1, 2, 8, 12, 22, 23, 24). Their causal nature consequently being excluded, the hypothesis as to their effect of the causal organism — a very favourite theme of the scientists — is sifted through as they are equally present in healthy plants. KLEBAHN (22) has recently tried to specify these bodies to certain virus diseases of potato. But being convinced of their non-organismic nature the terms „trypanoplasts" and „mastigoplasts" are designated to them by him.

Although no movement was observed by later investigators NELSON's observations regarding them was explained as due to changes „owing to the disturbances in osmotic pressure and other equilibria on emersion in water" (2).

Nothing can be said decisively about the nature of these bodies. They have been explained as „waxy bodies" made up of „elongated masses of gummy material long known to be characteristic of certain sieve tubes; cytoplasmic aggregations or areas of contraction possibly associated with disintegrating plastids; and homogenous aggregate of unknown origin possibly of waxy nature (12)"; „dexiotropic cell contents of an albuminoid nature (23)"; or „simple protein bodies" (1). The latter statement about their proteinous nature appears more assuring as they give positive tests for iodine, Biuret and Millons reagentia (1, 56).

Elytrosomes and Skolekosomes. The spindle shaped, elongated or fusiform bodies termed 'elytrosoma' were found in the mosaic-affected sugar beet (52). Though not exactly of the same form, very similar

bodies were discovered in the mosaic infected plants of bean (*Vicia faba*). These latter differ from those found by NELSON in not having the flagella. In the beet plant these bodies produced granules within them which are supposed to be formed by sporulation. The disease is thought to be transmitted by the ingestion and injection of these granules by aphids, as these bodies were also found in the intestines of those insects. Much doubt, however, is thrown on these results as such bodies have been found in healthy plants and described under the name of 'ooplasts' by KLEBAHN (22). The 'skolekosome' bodies found in Anemones suffering from „alloiophyllie" are out of consideration here as they were detected in healthy plants, though the disease producing monstrosity in those plants seems to be transmissible.

Other cytological investigations. The recent results arrived at by DUFRENOY (9, 10, 11) throw another light on the different aspects of these diseases. In the green portions of the leaves of *Phaseolus vulgaris* affected with mosaic no change in the conditions of plastids and mitochondria was observed. In the affected regions of light coloured patches he found the plastids not sensitive to staining and the starch grains absorbed. Much swelling and vacuolisation occurred in the plastids and mitochondria. Plasmolysis took place more easily in the affected cells than in the green ones. Similar conditions were found in leaves attacked by *Colletotrichum lindemuthianum*, an evidence which favours the conception that the malady is due to parasitism (9).

Another condition, produced in healthy plants by the action of hypotonic solutions, was found to be present in the mosaic diseased cells of the leaves of *Phytolacca decandra* and *Sida sp.* (10). At the same time a modification of plastids and mitochondria and the irregularity in the staining of vacuoles in the discoloured portions of the leaves in the same plants was noticed.

A close resemblance between the mosaic diseases of plants and animals has been shown by the same author (11). Whereas the small, filamentous vacuoles in healthy tissue swell and fuse to form a small number of large vacuoles, in the diseased part there is a large vacuome, surrounded by a number of small, filamentous vacuoles, condition similar to that found in the cellular inclusions of animal vaccines.

Lastly a mention of the work of Tsinen (57) should be made here. It was the first work done on the study of the mitochondrial cell inclusions in the chlorosis of plants. No surety can be made as to the

infectious nature of all the chlorosis of plants studied by him except that of *Abutilon Thompsoni*.

Various aspects of the question are as yet open to investigators, although scientists are certain that it has very little attraction from this side of the page. We may yet not despair about approaching the causal nature of the problem by explaining the mechanism of the phenomenon from its cytological radiations.

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DE BETEEKENIS DER CYTOLOGIE VOOR DE STUDIE VAN DE VIRUSZIEKTEN DER PLANTEN.

Een overzicht wordt gegeven van het cytologisch onderzoek van de virusziekten der planten, sedert den tijd dat IWANOWSKI zijn belangrijke, maar aanvankelijk weinig opgemerkte studie over het tabaksmozaiek publiceerde. Toen LYON vacuolen-lichaampjes bij de Fijiziekte van het suikerriet vond en KUNKEL dit onderzoek bij andere monocotylen voortzette, hebben tal van geleerden dezen weg gevolgd. Men heeft de vacuolen-lichaampjes nu gevonden zoowel bij virusziekten van vele monocotylen als bij die van vele dicotylen; wat men weet van hun structuur, deeling, encysteering, sporulatie, conjugatie, cysten, kieming en beweging is geschikt om de overtuiging te wekken, dat zij van dierlijken aard zijn en dat zij als de oorzaken van virusziekten moeten worden beschouwd. De geslachtsnaam *Vacuolarium* werd door LIKHITÉ voorgesteld in de plaats van vroeger gegeven geslachtsnamen.

De flagellate lichaampjes van ECKERSON worden als secundaire organismen beschouwd. Van extra lichaampjes gevonden bij Hippeastrum, suikerbiet en aardbei kent men de beteekenis nog niet; zij komen niet algemeen voor bij virusziekten.

Kristallijn materiaal is typisch voor „tobacco mosaic virus I" (JOHNSON). Dit materiaal wordt beschouwd als afscheidingsproduct van de vacuolen-lichaampjes; het is ook gevonden bij mozaiek en tusschennervig mozaiek van aardappel door KLEBAHN.

Van de flagellate en trypanosome-achtige lichaampjes gevonden door NELSON kan gezegd worden, dat het geen organismen zijn. KLEBAHN noemt ze „mastigoplasten" en „trypanoplasten"; microchemische reacties wijzen op een albuminoide natuur.

Of de zoogenaamde elytrosomen, door SCHAFFNIT en WEBER gezien in suikerbieten, beteekenis hebben voor de pathologie wordt betwijfeld, daar KLEBAHN dergelijke lichaampjes in gezonde planten heeft gevonden; hij noemt ze oöplasten. Ook de skolekosomen der anemonen vond hij in gezonde planten.

DUFRENOY heeft den toestand der zieke cellen van enkele plantensoorten vergeleken met die van gezonde cellen, welke in hypotonische oplossingen liggen. Of dit werk en dat van TSINEN beteekenis heeft voor de studie der virusziekten moet nog nader blijken.